



Atty Dkt. No.: CLON-056CIP
USSN: 09/858,332

Express Mail No.: EV462737750US

DECLARATION UNDER 37 C.F.R. §1.131 Address to: Commissioner for Patents P.O. Box 1450 Alexandria VA 22313-1450	Attorney Docket	CLON-053CIP
	Confirmation No.	2176
	First Named Inventor	Tchaga, Grigoriy S.
	Application Number	09/858,332
	Filing Date	May 15, 2001
	Group Art Unit	1652
	Examiner Name	Kathleen M. Kerr
	Title	<i>POLYNUCLEOTIDES ENCODING METAL ION AFFINITY PEPTIDES AND RELATED PRODUCTS</i>

Sir:

This Declaration and the attached Exhibit are being submitted in conjunction with the Applicants' Response to the Office Action dated May 19, 2004.

We, Grigoriy S. Tchaga, and George G. Jokhadze, do hereby declare as follows:

1. We are the listed co-inventors of the above-captioned application.
2. Enclosed with this declaration is Exhibits A that provides evidence of conception and reduction to practice of the claimed invention prior to July 1998.
3. Specifically, Exhibit A is an Invention Disclosure Form dated prior to July 1998 which describes the incorporation of a polyhistidine metal ion affinity sequences at the N- or C-terminal sequence of recombinant proteins and use for the purification of the recombinant proteins with high selectivity.
4. The disclosure appearing in Exhibit A clearly shows conception and reduction to practice of the claimed invention of the present application.

Atty Dkt. No.: CLON-056CIP
USSN: 09/858,332

5. Accordingly, the evidence provided in Exhibit A establishes that the claimed invention of the above captioned application was conceived and reduced to practice prior to July 1998.
6. We do hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date:

8/11/04

Grigoriy S. Tchaga

Grigoriy S. Tchaga

Date:

8/11/04

George G. Jokhadze

George G. Jokhadze

Attachment: Exhibit A

08/11/2004

17:10

CLONTECH LABS → 3273231

NO.124 0004

Atty Dkt. No.: CLON-056CIP
USSN: 09/858,332

Exhibit A



CLONTECH

INVENTION DISCLOSURE FORM

1. **Title of Invention:** _____
2. **Proposed Contributors to Invention.** (Please list each proposed contributor to the invention and summarize that proposed contributor's contribution to the invention).

Contributor #1	Grigoriy Simeonov	Contribution	
	Tchaga		
Contributor #2		Contribution	
Contributor #3		Contribution	
3. **Summary of the Invention.** Please provide a brief summary of the invention *e.g.* an abstract.

This invention describes the introduction of natural metal ion affinity sequences into recombinant proteins and the use of the novel metal ion affinity binding site for purification and/or immobilization of these proteins.
4. **Background of the Invention.** Please provide a discussion of the background of the invention. This discussion should include the following: (a) a discussion of the basic relevant technology;)b) a discussion of the problem solved by the invention and/or the benefits provided by the invention; and (c) a discussion of any relevant prior art of which you are aware. (For the prior art discussion, please provide citations and copies of relevant references, if at all possible).
5. **Detailed Description of the Invention.** Please provide a detailed description of the invention, including any relevant experiments which have been performed. In this section, please also include a detailed description of how the invention differs from the prior art and the advantages provided by these differences. In addition, please include a discussion of any variations of the invention which could work. (In this particular portion of the discussion, it may be helpful to think of yourself as a competitor and a contemplate how you would change the invention to achieve the same result in a different way.)

Introduction of Novel Metal Ion Affinity Site in Recombinant Proteins Based on Natural Polyhistidine Sequence

The polyhisidine sequence in question is a N-terminal peptide stretch from Lactate dehydrogenase from chicken muscle (*Gallus gallus*) encompassing 32 amino acid residues.

The affinity of the sequence for binding to immobilized Ni-ions (chelated with IDA ligand) has been determined after purification of the enzyme from a whole chicken

muscle extract by using the method of Tchaga (alias Chaga G. S.) et al (Ref?):

The purification procedure is based on the extremely high metal binding of the N-terminal region containing six histidine residues. Experiments performed both with the native LDH (tetramer of about 140 kD and the subunit of the enzyme (obtained after warming of the crude chicken muscle extract to 45 °C for 10 minutes) with molecular size of 36388 D show that the retention of the enzyme on immobilized Ni is not cooperative, both the native protein and the subunit are adsorbed at imidazole concentration up to 60 mmol and eluted completely at 300 mmol imidazole concentration.

The enzyme was subjected to CNBr cleavage and the mixture of the peptides was applied to immobilized Ni-IDA column with metal ion capacity of 32 μ mol per mL gel.

The loading conditions were the same as those used for the purification of the enzyme from the crude extract. The adsorbed material was eluted again with 300 mmol imidazole. It was further subjected to RPC chromatography and the peak containing about 80 % of the adsorbed material was subjected to amino acid analyses. The results point out that this peak corresponds to the N-terminal CNBr cleavage product from LDH, containing the polyhistidine sequence.

The fact that the peptide retained its binding affinity even after treatment with CNBr in presence of 70 % TFA is ample proof that the binding is not contributed to a rigid secondary conformation structure.

This result is also in agreement with the experimental data showing that only one polypeptide chain (subunit) is responsible for the very strong binding of the enzyme to immobilized Ni-ions.

The cDNA of this polypeptide is incorporated as N- or C-terminal sequence of recombinant proteins and used for the purification of the recombinant proteins with high selectivity. The presence of this sequence in recombinant proteins provides them with novel affinity for immobilized metal ions. Since the strength of binding is very high, the purification procedure for such proteins is very selective and at the same time the capacity of the adsorbents used for purification is very high towards the protein of interest. As a consequence, there is lower demand for the amount of adsorbent. Incorporation of proteolytic site of highly specific protease between the binding sequence and the native sequence of the protein delivers the means to produce the native protein. This is achieved after limited proteolysis and a second chromatography step in which the proteolytic product is loaded on the same immobilized metal ion affinity column and the native protein passes through the column without retardation, where as the metal ion binding sequence is adsorbed on the column. Additional improvement on ease and increased recovery is the incorporation into the cDNA of secretion signal that causes the protein of interest to be secreted into the fermentation media after synthesis. Since a considerable amount of the protein remains in the cell, this improves dramatically the following isolation and purification by eliminating the need of cell disruption, extraction and removal of unwanted cell components and DNA/RNA.

Another important application of this sequence is for the immobilization of the proteins containing it. Immobilized products based on this principle carry the advantages of a very strong attachment combined with reversible nature of the

immobilization process, ability to regenerate and reuse the carrier multiple (more than 100) times after the inactivation of the immobilized proteins. Another important feature of the immobilized preparations is the very high probability that the immobilized recombinant proteins will retain full biological activity and specificity due to the utilization only of the additional metal binding site in the immobilization process but not of a part of the native protein structure. This, again, is ensured by the highly discriminatory (for non specific binding) imidazole concentration present during the immobilization process. In practice it means that for the preparation of immobilized proteins there is no need for purification of the protein preceding the immobilization procedure because the immobilization process carries over also a purification effect. Such a combination is important for achievement of high operational capacity.

This

6. **Relevant Dates**

Please fill in each of the following sections:

a. **First date on which Invention was Conceived and/or demonstrated/tested.**

Please provide details as to the exact date, time and location of: (a) when the invention was conceived; and (b) the first demonstration or test of the invention. Also please provide a detailed description of the test(s) performed, and give names and addresses of any witnesses present during the demonstration or test.

The date I came for the interview - conceiving????

First demonstration - is it in the future????

b. **First date of disclosure of Invention with Clontech.** Please provide details as to the exact date and time of the first internal disclosure, the circumstances of the disclosure and to whom the disclosure was made.

c. **First date of disclosure of Invention outside Clontech.** Please provide details as to the exact date and time of the first external disclosure, the circumstances of the disclosure and to whom the disclosure was made.

d. **Proposed or past publication and commercialization dates.** Please provide a detailed description of any activity that might constitute publication of the Invention, advertising of the Invention or placing the Invention on sale. If no such activity has yet taken place, please provide an estimated date as to when any such activity might take place.

7. **Related Government or Other Contract Information.** Examples of such information include information about any relevant government grants which may have funded the Invention, in whole or in part, and the like.

8. **Contributors**

- (1) Grigoriy Simeonov Tchaga

(Name)

(Signature)

(Date)

- (2)

(Name)

(Signature)

(Date)

- (3)

(Name)

(Signature)

(Date)

9. **Witnesses**

(Witnesses must understand the significant details of the invention and must appreciate the factors which enable successful functioning of the invention for its intended purpose. A co-inventor cannot be a witness).

Witness Statement

I have read and understand this Invention Disclosure.

- (1)

(Name)

(Signature)

(Date)

- (2)

(Name)

(Signature)

(Date)